# Absence of hemolytic disease of fetus and newborn despite maternal high-titer IgG anti-Ku

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Anti-Ku seen in $K_o$ (Kell-null) individuals has previously been shown to cause severe hemolytic transfusion reactions. Maternal anti-Ku can cause none or moderate to severe hemolytic disease of the fetus and newborn (HDFN). In two of four previously described HDFN cases, intrauterine transfusions were required because of severe anemia. We report a case in which maternal anti-Ku did not cause HDFN. Standard serologic methods were used for RBC antibody screening and identification, adsorption and elution of RBC antibodies, and antigen typing. A gravida 3, para 3 (G3P3) woman was first evaluated in 2006 and was found to have an IgG RBC antibody that reacted against all panel RBCs in the anti-human globulin phase. A panel of RBCs treated with DTT did not react with the antibody. The antibody failed to react with one example of $K_o$ RBCs. The patient's RBCs typed negative for the following Kell blood group antigens: KEL1, KEL2, KEL3, KEL4, KEL6, KEL7, KEL11, KEL13, and KEL18. These results established the presence of anti-Ku in maternal serum. The newborn was group A, D+ and required phototherapy for hyperbilirubinemia, but did not require transfusion. The woman was seen again in January 2010 during the third trimester (G4P3). At this time, anti-Ku titer was 256. She delivered a healthy group O, D+ baby boy at 37 weeks' gestation. Cord RBCs were 4+ for IgG by DAT. An eluate reacted with all RBCs tested, but did not react when tested against a panel of DTT-treated RBCs. $K_o$ phenotype is rare to begin with, and the maternal anti-Ku formation may require more than one pregnancy. Therefore, cases that can be evaluated for anti-Ku–related HDFN are rare. Our case contributes to serologic and clinical aspects of such rare cases. ***Immunohematology* 2010;26:119–122.**

**Key Words:** Kell blood group, anti-Ku, Kell-null, $K_o$, hemolytic disease of fetus and newborn, maternal alloimmunization, newborn hyperbilirubinemia, autologous blood donation, immunohematology

The Kell blood group system is polymorphic and is known to contain both low- and high-prevalence antigens. KEL1 (synonyms K, Kell), KEL3 (synonym $Kp^a$), and KEL6 (synonym $Js^a$) are examples of low-prevalence antigens, and KEL2 (synonyms k, Cellano), KEL4 (synonym $Kp^b$), and KEL7 (synonym $Js^b$) are high-prevalence antigens. An antithetical relationship between some of the low- and high-prevalence antigens, for example, KEL3 and KEL4, has been described. Kell system antibodies directed toward the low- and high-prevalence antigens are known to be clinically significant. Antibody against a high-prevalence antigen KEL5 (synonym Ku) is made by individuals with $K_o$ RBCs that lack all Kell antigens, i.e., are $K_o$ (Kell-null). Other examples of high-prevalence antigens include KEL11, KEL12, KEL13, KEL14, KEL16, KEL18, KEL19, KEL20, and KEL22. Kx antigen is phenotypically related to the Kell blood group, but is coded by a gene located on the X chromosome. The Kell blood group system was reviewed by Westhoff and Reid.[1]

For routine blood banking, commercial antibodies directed toward KEL1, KEL2, and KEL3 are available for patient and donor typing. Commercial panel RBCs characterized for these antigens and others, such as KEL4, KEL6, and KEL7, are available for antibody detection and identification. Kell blood group antigens are inactivated by treatment of RBCs with 2-aminoethylisothiouronium (AET) or DTT. This characteristic is valuable in the identification of Kell antibodies.

Kell blood group antibodies are known to cause anemia of the fetus and newborn (AFN). In one study of 311 KEL1-alloimmunized pregnant women who had 459 pregnancies, 376 infants were not affected at birth but 20 were affected.[2] The 376 unaffected infants were KEL1–except for rare cases for which paternal or cord blood sample was not available for investigation. Of the affected infants, 12 did not require treatment although their RBCs were positive by the DAT; 4 needed exchange transfusion, phototherapy or both; 1 had moderately severe disease requiring multiple exchange transfusions; and 3 had very severe disease requiring fetal transfusions, or were hydropic or stillborn. In this report, 4 infants with severe fatal AFN were observed in the remote past (1948–1954).[2] These data show that most maternal anti-KEL1 do not cause AFN.

As noted previously, some maternal anti-KEL1 can cause AFN. Most frequently these antibodies cause fetal anemia through suppression of fetal erythropoiesis. Hemolysis and erythropoiesis suppression together can cause severe anemia in the fetus and the newborn. Kell antigens are present on erythroid, myeloid, and megakaryocytic precursors. As a result, maternal anti-KEL1 can cause severe fetal anemia and also severe thrombocytopenia.

In the general population, absence of phenotypic expression of Kell antigens is rare, and this phenotype is referred to as $K_o$. Individuals with the $K_o$ phenotype can form antibodies that react with RBCs from all individuals except those who are $K_o$. Such antibodies are referred to as anti-Ku (KEL5). Anti-Ku can cause hemolysis if patients are transfused with incompatible RBCs.[3] In fact, a fatal hemolytic transfusion reaction after transfusion of 34 units of

incompatible RBCs has been described.[4] In addition to the hemolytic transfusion reaction, maternal anti-Ku has been described as the cause of AFN in four patients.[5–8] Additionally, lack of AFN from such antibodies has also been observed previously (personal communication from Marion Reid, April 4, 2010). The first case of AFN from anti-Ku was reported by Corcoran et al. in 1961.[9] The details regarding the degree or the severity of hemolysis and its effect on the fetus or newborn were not included in the report. Absence of AFN from maternal anti-Ku has been noted previously.[10] Because the $K_0$ phenotype is rare and women with this phenotype must be alloimmunized with perhaps more than one pregnancy, finding such rare cases to study is difficult. Therefore, in this report we describe our case in which maternal anti-Ku did not cause AFN.

## Material and Methods

Testing for ABO, Rh, and Kell system antigens was performed with commercially available reagents (ABO, Rh, KEL1, KEL2, and KEL3 typing sera were from ImmucorGamma Inc., Norcross, GA) by the tube method. In-house, single-donor source anti-KEL4, anti-KEL6, anti-KEL7, anti-KEL11, anti-KEL13, and anti-KEL18 were used to type for respective Kell antigens. Antibody screening and identification methods used tube testing with commercially available reagent RBCs and reagents (LISS and PEG from ImmucorGamma). Maternal serum was tested against commercially available ficin-modified RBCs in which serum and RBCs were incubated at 37°C for 15 minutes followed by testing with anti-human globulin (AHG). Adsorption of anti-Ku was carried out by incubation of maternal serum with KEL1+ and KEL1– RBCs at 37°C for 30 minutes and the absorbed serum was subsequently tested by the tube method. Titration of anti-Ku was performed by incubating serial saline dilutions of the serum with saline-suspended RBCs at 37°C for 60 minutes followed by testing with anti-human globulin. Titer was assigned to the highest dilution which resulted in macroscopic reactions. Eluate from cord RBCs was prepared by using commercially available kit (Elu-Kit II, ImmucorGamma) according to manufacturer's instructions. DTT treatment of RBCs was accomplished by adding one volume of a 50% RBC suspension to an equal volume of 0.01M DTT solution; the mixture was then incubated at 37°C for 15 minutes. The excess DTT was removed by washing the treated RBCs 4 times with saline.

## Case Report and Results

A 35-year-old previously nontransfused woman from Ecuador, gravida 3, para 3 (G3P3), was first referred to our reference laboratory in February 2006. At this time she had delivered her third baby at a local hospital. On routine workup on a sample from the labor and delivery floor, she was found to be group O, D+, and on antibody screening, she was found to have an antibody that reacted with RBCs in the AHG phase. The baby's blood typed as group A, D+. Maternal sample testing was completed at our laboratory, and the findings are shown in Table 1. The titer of the maternal antibody was not determined.

The serologic findings in 2006 described previously and in Table 1 demonstrated that the mother was $K_0$ and had anti-Ku in her serum. The baby was group A, D+ and required phototherapy after birth, most likely owing to maternal anti-A. However, the details about the baby were not available. The mother indicated that this baby did not require any transfusions during pregnancy or after delivery. The mother also reported that the first two babies were born in Ecuador and neither of them had required any phototherapy or transfusions.

This patient presented again in January 2010 at approximately 35 weeks of gestation with her fourth pregnancy (G4P3). A prenatal blood sample obtained at this time was sent to our laboratory for workup. The maternal sample typed as group O, D+. Serologic findings are shown in Table 2.

**Table 1.** Serologic findings for maternal sample in 2006

| Procedure | Results | Interpretation/ comment |
|---|---|---|
| Maternal serum studies | | |
| Nonenhanced panel | Negative at IS and 37°C Positive reactions (2+ to 4+) with all panel RBCs at AHG | Pan-reactive antibody |
| LISS panel | Positive at IS, 1+ at 37°C, and 3+ to 4+ at AHG with all panel RBCs | Pan-reactive antibody with stronger reactions at AHG |
| Ficin enzyme panel | 1+ at 37°C and 3+ at AHG with all RBCs | Pan-reactive antibody at 37°C and at AHG |
| DTT-treated panel with LISS | No reactions at AHG with all six panel RBCs | Findings suggest antibody specificity may reside in Kell group system |
| Maternal serum adsorbed with KEL1+ RBCs tested in a LISS panel | No reactions at IS, 37°C, and AHG | Adsorption with KEL1+ RBCs removed the antibody |
| Maternal serum tested against $K_0$ RBCs | Negative reaction | Antibody does not react with $K_0$ RBCs. Findings suggest presence of anti-Ku |
| Maternal RBC studies | | |
| RBC typing for Kell group antigens | KEL:–1,–2,–3,–4,–6, –7,–11,–13,–18 | Low- and high-prevalence antigens absent; in-house antisera used for typing except for KEL1, KEL2, and KEL3 |
| RBC typing for other antigens | D+,C+,E+,c+,e+; Fy(a–b+); Jk(a–b+); M+,N–,S–,s+ | $R_1R_2$ phenotype |

AHG = anti-human globulin; IS = immediate spin.

A fetal ultrasound performed at 36 weeks and 1 day's gestation demonstrated the peak systolic velocity of 0.52 m/s on the middle cerebral artery Doppler, which is normal. A follow-up ultrasound to rule out hydrops fetalis or fetal anemia was also performed 4 days later and showed normal peak systolic velocity of 0.44 m/s. In addition, there were no signs of hydrops fetalis.

**Table 2.** Maternal and cord blood sample serologic testing results in 2010

| Procedure | Results | Interpretation/ comment |
|---|---|---|
| Maternal serum testing | | |
| LISS panel | Positive to 1+ at IS, + to 1+ at 37°C, and 4+ at AHG with all panel RBCs | A panreactive antibody with stronger reactions at AHG |
| DTT-treated, ficin-treated, and PEG panels | Negative reactions with all panel RBCs | Antibody not reactive with DTT-treated RBCs suggests Kell group related antibody. Kell antigens are generally considered resistant to ficin. So, negative reactions with ficin-treated RBCs remain unexplained. Antibody nonreactive in PEG. |
| Titer against KEL1+ RBCs | 256 | High-titer antibody |
| Titer against KEL1– RBCs | 256 | |
| Maternal serum tested against $K_0$ RBCs | Negative reaction | Antibody does not react with $K_0$ RBCs. Findings suggest presence of anti-Ku |
| Cord RBC testing | | |
| Blood group/D typing | O, D+ | |
| Rh phenotype | C+,c+,E+,e+ | $R_1R_2$ phenotype |
| DAT | 4+ IgG | Positive DAT |
| DAT on chloroquine-treated RBCs | Positive | Unable to remove bound antibody to perform phenotyping of Kell group antigens |
| Cord serum testing | | |
| Serum screening with LISS and gel | Negative reactions | Absence of antibody in the serum |
| Eluate from cord RBC testing | | |
| Eluate tested with nonenhanced panel RBCs | 3+ at AHG with all panel RBCs | Panreactive antibody |
| Eluate tested with DTT-treated panel RBCs | No reactions | Abolition of antibody reactivity with DTT-treated RBCs suggests Kell group specificity |

AHG = anti-human globulin; IS = immediate spin.

The mother donated a unit of whole blood at 37 weeks of gestation for her own use and also for her baby if needed after the birth. The mother went into spontaneous labor the day after the blood donation at 37 weeks of gestation and delivered a normal healthy boy. At birth, the baby's Hb level was 22.7 g/dL, and the total bilirubin was 1.3 mg/dL. The baby's Hb levels on day 1 were 20.7 and 21.1 g/dL. On days 2 and 3, these values were 17.5 and 18.2 g/dL, respectively. The baby's total bilirubin values are shown in Table 3. The highest total bilirubin, 15.4 mg/dL, was seen on day 3. It should be noted that for term newborns, the upper 95th percentile total bilirubin at 72 hours is 16.0 mg/dL.[11] At this point, the baby was briefly treated with phototherapy and discharged home on day 5 in good condition.

A cord blood sample was tested in our laboratory, and the baby was found to be group O, D+. Cord RBCs and serum testing results are shown in Table 2.

**Table 3.** Newborn's total bilirubin levels

| Day | Time | Total bilirubin (mg/dL) |
|---|---|---|
| 1 | Cord blood at 15:15 | 1.3 |
| | 16:50 | 1.6 |
| | 21:32 | 3.0 |
| 2 | 9:12 | 5.7 |
| | 10:06 | 5.7 |
| 3 | 10:00 | 11.9 |
| | 16:28 | 14.4 |
| | 21:36 | 15.4 |
| 4 | 04:16 | 13.6 |
| | 10:28 | 13.4 |
| | 16:15 | 12.8 |
| 5 | 00:25 | 12.2 |
| | 08:05 | 13.1 |

## Discussion

Anti-Ku is seen in individuals who are $K_o$. Anti-Ku reacts with all RBCs except those that are $K_o$. $K_o$ RBCs do not react with the antisera directed against other Kell system antigens, such as KEL1, KEL2, KEL3, KEL4, etc. In our case, maternal RBCs were tested for the presence of several Kell antigens, namely KEL1, KEL2, KEL3, KEL4, KEL6, KEL7, KEL11, KEL13, and KEL18, and all were found to be lacking. Maternal serum in our case also showed reactions with all panel RBCs tested with LISS enhancement, and these reactions were abolished when tested against a DTT-treated panel. The maternal serum also did not react against cells from a $K_o$ individual. These findings support the conclusion that the woman in our case was $K_o$ and had developed anti-Ku. A Ku-like antibody has been described in individuals whose RBCs are classified as $K_{mod}$. RBCs with the $K_{mod}$ phenotype give weak reactions with antisera directed against Kell antigens.[12] In our case, maternal RBCs failed to give any reactions with such antisera, supporting the conclusion that the woman in our case was $K_o$ and not $K_{mod}$.

We are aware of four published cases of anti-Ku–mediated AFN.[5–8]. One of these reports is published in a non-English journal, and we were unable to obtain the details of the case.[5] The other three cases show severe AFN. For instance, a case of AFN has been described in which the newborn developed anemia with a Hb of 10.7 g/dL 10 hours after birth and the baby's bilirubin was 18.1 mg/dL.[6] The baby was given three exchange transfusions of KEL: –1 blood. Further studies showed that the mother had the phenotype $K_o$ and the baby's phenotype was KEL: –1,2, –3,–4,–6, 7. The mother had high-titer anti-Ku. In two other cases, intrauterine transfusions of RBCs were needed.[7,8] In one of the two cases, the fetus required regular intrauterine transfusion to manage severe anemia with a Hb of 3 g/dL.[7] In this case, the mother was also treated with recombinant erythropoietin. A baby boy was delivered at 36 weeks of gestation with a Hb of 15.8 g/dL. After birth, the baby developed only mild hyperbilirubinemia. On the 15th day of life, the infant's Hct was 27.3%; both his reticulocyte count and erythropoietin level were low. The infant was subsequently treated with recombinant erythropoietin. In the second case that required intrauterine RBC transfusions, a pregnant 30-year-old woman was first seen at 18 weeks of gestation and was found to have anti-Ku following one transfusion, one pregnancy, and two abortions.[8] She was of the $K_o$ phenotype. The titer of the antibody ranged from 1024 to 4096 during the pregnancy. At the 26th week of gestation, the fetus was found to have hydrops fetalis and underwent emergency intrauterine transfusion with maternal RBCs. By 35 weeks of gestation, while receiving recombinant erythropoietin, the mother donated four units of RBCs for four additional fetal intrauterine exchange transfusions. The baby was delivered by cesarean section, required phototherapy but did not need exchange transfusion.

As described previously, in our case, there was no clinical evidence for AFN even though the mother had IgG anti-Ku and the newborn cord RBCs had a positive DAT with anti-IgG and an eluate that reacted with all RBCs tested but not with DTT-treated RBCs. Maternal antibody titer in our case was 256 and is less than in the previously reported case described earlier. As our case illustrates, anti-Ku titer in itself may not be predictive of AFN (which is typical of Kell antibodies). The fetus in our case had a carotid artery Doppler study that showed absence of anemia. At birth, cord blood hemoglobin and bilirubin values were normal. A mild elevation in total bilirubin was seen on the third day of life, which was treated with phototherapy, and the newborn was discharged in good condition on day 5. The lack of AFN in our case could be attributable to an anti-Ku IgG subclass. For instance, IgG2 and IgG3 subclass antibodies are not transported across the placenta as readily as IgG1 and IgG4. Our laboratory is not able to perform subclass determination of IgG antibody, and therefore such studies were not performed. A monocyte monolayer assay to assess the clinical significance of maternal anti-Ku would have been helpful, but these latter studies were not performed.

The lack of AFN with anti-Ku has been observed previously (personal communication from Marion Reid). Our case contributes valuable information on serologic and clinical findings about the lack of AFN from anti-Ku in view of the fact that $K_o$ individuals are rare, the opportunities to study AFN from anti-Ku are rare, and the publication bias is generally toward reporting those cases in which infants are affected.

## Acknowledgments

The authors wish to thank Patricia Bradford, MT(ASCP), and Jeffrey Lee, MT(ASCP)SBB, for their technical assistance in performing the serologic testing.

## References

1. Westhoff CM, Reid ME. Review: the Kell, Duffy, and Kidd blood group systems. Immunohematology 2004;20:37–49.
2. Bowman JM, Pollock JM, Manning FA, Harman CR, Menticoglou S. Maternal Kell blood group alloimmunization. Obstet Gynecol 1992;79:239–44.
3. Daniels G. Kell and Kx blood group systems. In: Daniels G. ed. Human blood groups. 2nd ed. Oxford, UK: Blackwell Science Ltd, 2002:304.
4. Lin M, Wang CL, Chen FS, Ho LH. Fatal hemolytic transfusion reaction due to anti-Ku in a K null patient. Immunohematology 2003;19:19–21.
5. Huang HJ, Tagawa H. Hemolytic disease of the newborn due to anti-Ku [in Japanese]. Nippon Sanka Fujinka Gakkai Zasshi 1982;34:119–21.
6. Fourmaintraux A, Vitrac D, Mariette JB, Brunel F. The $K_o$ phenotype and fetal-maternal alloimmunization [in French]. Arch Fr Pediatr 1993;50:779–81.
7. Manoura A, Korakaki E, Hatzidaki E, et al. Use of recombinant erythropoietin for the management of severe hemolytic disease of the newborn of a $K_o$ phenotype mother. Pediatr Hematol Oncol 2007;24:69–73.
8. Lydaki E, Nikoloudi I, Kaminopetros P, et al. Serial blood donations for intrauterine transfusions of severe hemolytic disease of the newborn with the use of recombinant erythropoietin in a pregnant woman alloimmunized with anti-Ku. Transfusion 2005;45:1791–5.
9. Corcoran PA, Allen FH Jr, Lewis M, Chown B. A new antibody, anti-Ku (anti-Peltz), in the Kell blood-group system. Transfusion 1961;1:181–3.
10. Reid ME, Lomas-Francis C. The blood group antigen factsbook. 2nd ed. San Diego, CA: Academic Press, 2004.
11. Subcommittee on hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004; 114:297-316.

12. Lee S, Russo DC, Reid ME, Redman CM. Mutations that diminish expression of Kell surface protein and lead to the $K_{mod}$ RBC phenotype. Transfusion 2003;43:1121–5.

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